DOCUMENT RESUME

ED 179 413 SE 029 436

AUTHOR	Friedland, James
TITLE	Huntington II Simulation Program - PH. Student Workbook, Teacher's Guide, and Resource Handbook.
INSTITUTION	Digital Equipment Corp., Maynard, Mass.; State Univ. of New York, Stony Brook. Huntington Computer Project.
SPONS AGENCY	National Science Foundation, Washington, D.C.
PUB DATE	Oct 73
GRANT	NSF-GW-5883
NOTE	62p.; For related documents, see SE 029 434-440 and ED 093 644-645; Not available in hard copy due to marginal legibility of original document
EDRS PRICE	MF01 Plus Postage. PC Not Available from EDRS.
DESCRIPTORS	*Biology; *Chemistry; *Computer Assisted Instruction; Game Theory; Mathematics Education; Models; *Physiology; *Science Education; Secondary Education; *Simulation
IDENTIFIERS	*Enzymes

ABSTRACT

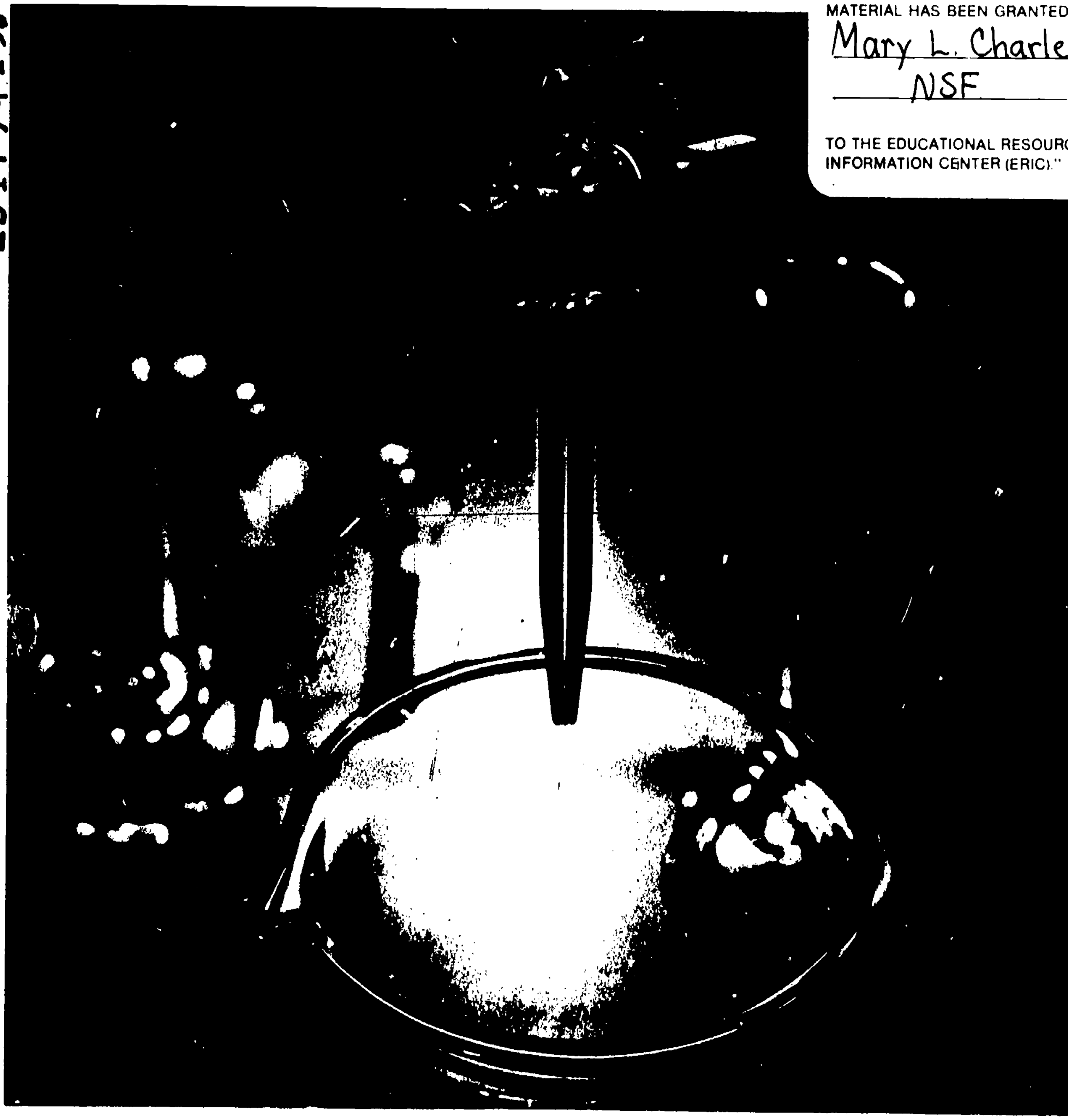
Described is the computer simulation program "PH." The program consists of three different laboratory investigations dealing with the pH specificity of enzymes. The purpose of the program is to enable tenth- to twelfth-grade students to determine a possible explanation for pH specificity in an experimental, but mathematical, fashion. (Author/RE)

DIGITAL EQUIPMENT CORPORATION

STUDENT WORKBOOK $ 50

education

Huntington II Simulation Program - PH



digital

DIGITAL EQUIPMENT CORPORATION
EDUCATIONAL PUBLICATIONS

A partial list of the publications in the continuing series of curriculum material published by Digital for use with EduSystems and RSTS are listed below. Please inquire directly for prices on classroom quantities.

These publications may be ordered (cash enclosed) from:

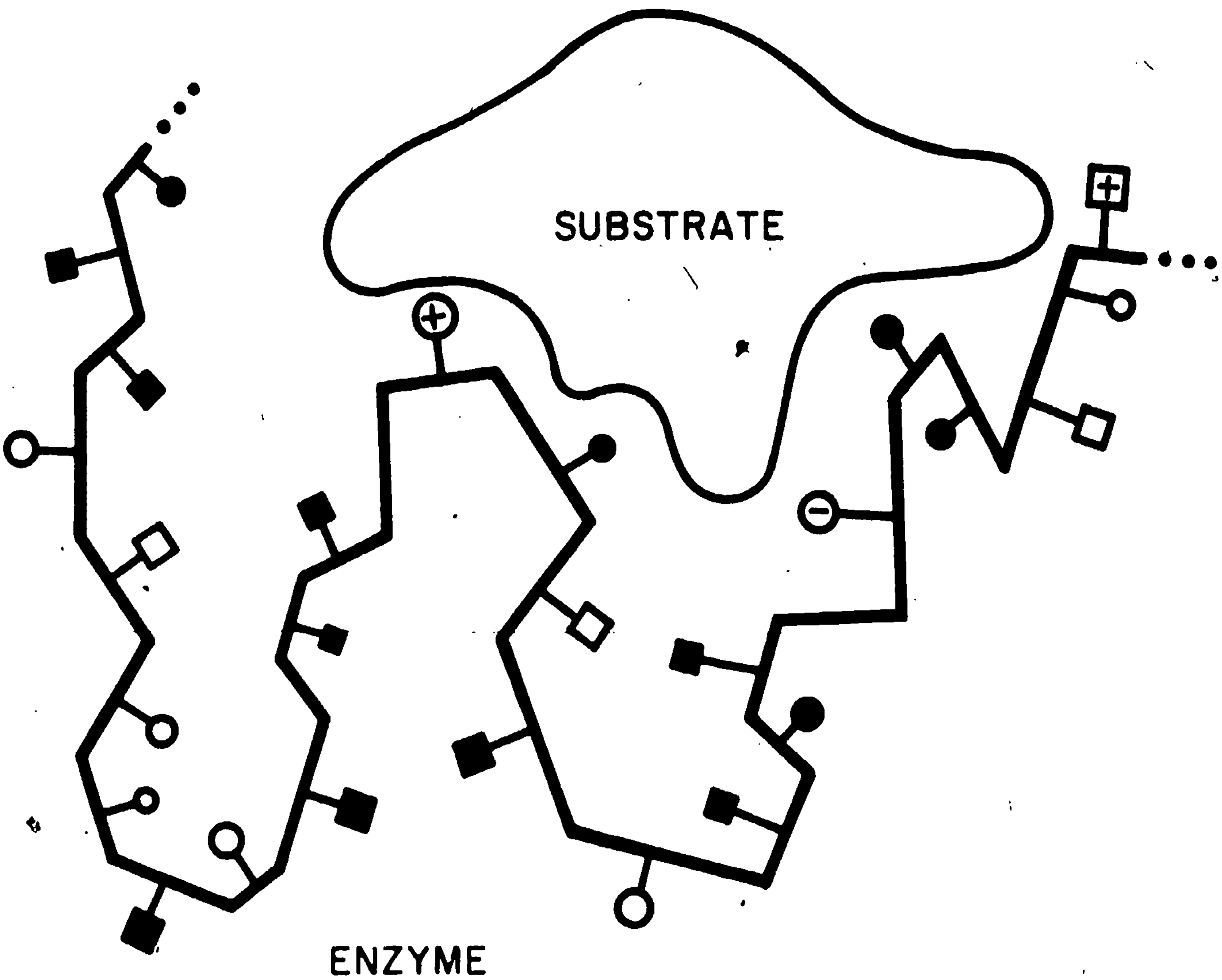
Software Distribution Center
Digital Equipment Corporation
Maynard, Massachusetts 01754

Population: Self Teaching BASIC Workbook		$2.00
101 BASIC Computer Games		$5.00
Understanding Mathematics and Logic Using BASIC Computer Games		$1.50
BASIC Matrix Operations, Project Solo		$1.00
Computer Augmented Calculus Topics		$1.50
Problems for Computer Mathematics		$1.25
Advanced Problems for Computer Mathematics		$2.00
Tutorial Exercises for Chemistry	Student Workbook	$1.00
	Teacher's Resource	$2.75
BASIC Application Programs		
Mathematics I		$1.00
Mathematics II		1.00
Science		1.00
Business and Social Studies		1.00
Plotting		1.00
Chemistry		1.50
Huntington I Application Programs		
Mathematics		$2.00
Teacher Assistance		1.00
Huntington I Simulation Programs		
Biology		$1.00
Chemistry		2.00
Earth Science		1.00
Physics		2.00
Social Studies		1.00
Huntington II Simulation Modules		
Student Workbook		$.30 - .50
Teacher's Guide		.30 - .50
Resource Handbook		.50 - 1.00
Program Paper Tape		.50 - 1.00

STUDENT MANUAL

PH

THE PH SPECIFICITY OF ENZYMES



HUNTINGTON TWO COMPUTER PROJECT

14 October 1973

The work of the Huntington Two Computer Project is partially supported by the National Science Foundation, Grant GW-5883.

PUBLISHED EXCLUSIVELY BY DIGITAL EQUIPMENT CORPORATION, 1973

STUDENT MANUAL

I. INTRODUCTION

Enzymes are pH specific; that is, they work best at certain pH values. For example *pepsin*, the enzyme that digests protein in your stomach, works well at pH values of 1 and 2 but doesn't work at all at pH 9. Why?

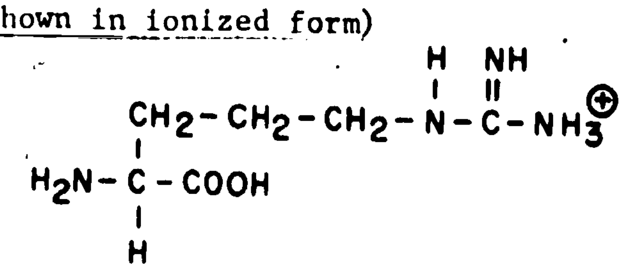
Scientists have not yet agreed on an answer to this question, but there are several theories. The *PH* program allows you to explore one of these theories without having to do the required math.

The theory that the *PH* program is based on holds that pH specificity can be traced to the behavior of ionizable amino acids present at the active site. By *ionizable* we mean that the amino acids can become charged. The *active site* is the particular location on the enzyme where catalysis occurs.

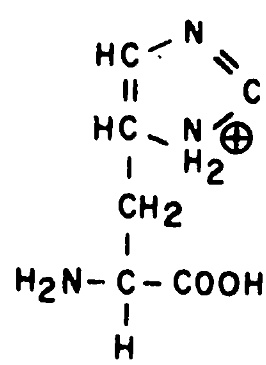
Studies of many different enzymes indicate that there are usually ionized amino acids present at an active site. Amino acids that take a positive (+) charge are called *basic* amino acids, those that take a negative (-) charge acidic amino acids. There are only a few of each kind. Some of these are listed below:

BASIC AMINO ACIDS (shown in ionized form)

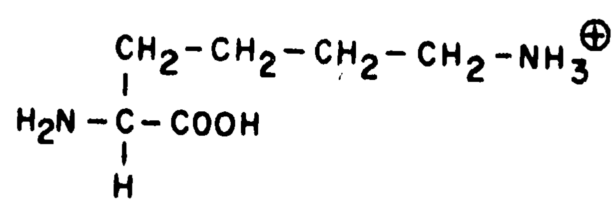
ARGININE



HISTIDINE

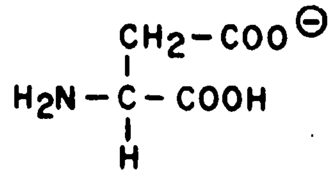


LYSINE

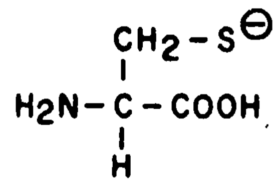


ACIDIC AMINO ACIDS (shown in ionized form)

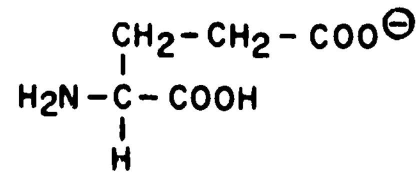
ASPARTIC ACID



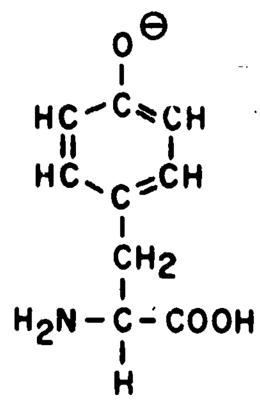
CYSTEINE



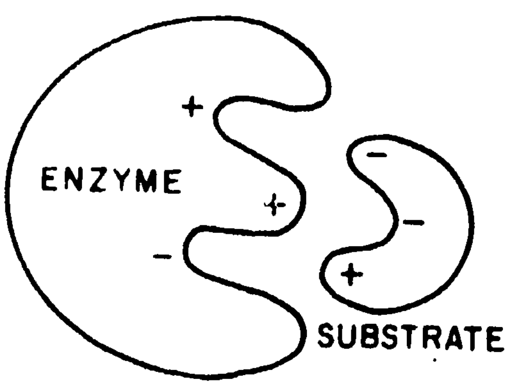
GLUTAMIC ACID



TYROSINE



The ability of an amino acid to become charged can be very important to enzyme activity if, for example, it is the attraction of opposite charges that is responsible for holding the enzyme's *substrate* (the substance the enzyme works on) in correct position on the surface of the enzyme during catalysis.



As we said before, pH specificity can probably be traced to the behavior of ionizable amino acids present at the active site, the explanation for this being the different pH values at which different amino acids become charged. Does it matter how many amino acids are present at a site? Does the type of amino acid make any difference? These are some of the questions you will try to answer with the *PH* program.

INVESTIGATION #1 *PATTERNS OF ENZYME ACTIVITY*

The *PH* model predicts high enzyme activity when the active site is properly charged and low enzyme activity when there is an improper charge at the active site. As you have already read, the ionizable amino acids become charged *only at certain pH values*, some at high pH only and some at low pH only. Through this investigation you should be able to figure out at what pH each of the seven ionizable amino acids is charged.

Experiment 1 — WHAT PATTERN OF ENZYME ACTIVITY WOULD BE EXPECTED IF AN ENZYME HAD ONLY ONE CHARGED ACIDIC AMINO ACID AT ITS ACTIVE SITE?

To answer the question it will be necessary to run *PH* at least once and possibly several times. If you find it necessary to run *PH* more than once, remember that in a controlled experiment only one thing is varied from experiment to experiment, all other variables remaining constant.

The program will first ask you for a series of inputs:

HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE?

The question above gives you the answer to this question. You may want to enter this answer on an INPUT SHEET (see end of this Manual).

The computer will next ask:

AMINO ACID 1 ---- CODE NO.?

These are the code numbers for each of the acids:

1 = aspartic acid
2 = cysteine
3 = glutamic acid
4 = tyrosine
5 = arginine
6 = histidine
7 = lysine

Numbers 1 through 4 are acidic amino acids, while numbers 5 through 7 are basic amino acids.

SHOULD IT BE CHARGED (1=YES, Ø=NO)?

We assume in this experiment that the amino acid must be in the charged condition for enzyme activity.

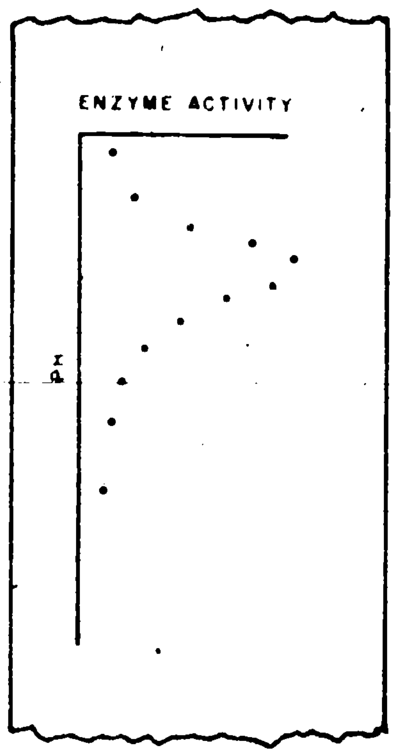
PH RANGE FOR THIS EXPERIMENT:

LOWER LIMIT?
UPPER LIMIT?

Since we are interested in enzyme activity over the entire pH range, you should select your limits accordingly. (You are limited to the range from 0 to 14.)

NOTE: It would be a good idea for you to plan your experiment and record your inputs before you go to the computer. You can use [illegible] the COMPUTER INPUT SHEETS included at the back of this [illegible].

When you have finished entering your inputs, the computer will carry out the necessary calculations and then give you the results in graphical form. Enzyme activity will be plotted on the y-axis, and pH on the x-axis. Remember that a computer graph always comes out *sideways*, so you will have to turn it on its side for easier reading.



AS IT COMES OFF THE TELETYPE

TURNED OVER FOR READING

Now, see if you can answer the following question:

OVER WHICH pH RANGE, FROM 0-7 OR 7-14, WOULD AN ENZYME THAT REQUIRES THE CHARGED ACIDIC AMINO ACID WORK BEST?

Experiment 2 — WHAT ENZYME ACTIVITY PATTERN WOULD BE EXPECTED FOR AN ENZYME THAT REQUIRES ONE CHARGED BASIC AMINO ACID AT THE ACTIVE SITE FOR ENZYME ACTIVITY?

If you did not carry out Experiment 1, please read Experiment 1 instructions first.

Since the only change for Experiment 2 is in the type of ionizable amino acid present, your inputs should differ for *only one* question.

AMINO ACID 1 ---- CODE NO.?

Amino acids coded 5 – 7 are basic amino acids.

Again, be sure to record your inputs on a COMPUTER INPUT SHEET before going to the machine.

At the end of this experiment you should be able to answer the question:

OVER WHICH pH RANGE, 0-7 OR 7-14, SHOULD AN ENZYME THAT REQUIRES ONE CHARGED BASIC AMINO ACID WORK BEST?

Experiment 3 — WHAT PATTERN OF ENZYME ACTIVITY WOULD BE ASSOCIATED WITH AN ENZYME THAT REQUIRES TWO CHARGED AMINO ACIDS AT THE ACTIVE SITE, ONE ACIDIC AND ONE BASIC?

If you did not carry out Experiment 1, be sure to read the instructions starting on page 3 before going on.

For this experiment you will have to enter inputs for the following questions:

- HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE?
- AMINO ACID 1 ---- CODE NO.?

  SHOULD IT BE CHARGED (1=YES, Ø=NO)?
- AMINO ACID 2 ---- CODE NO.?

  SHOULD IT BE CHARGED (1=YES, Ø=NO)?
- PH RANGE FOR THIS EXPERIMENT?

  LOWER LIMIT?
  UPPER LIMIT?

Since there are many possible combinations of amino acids that can be used in this experiment, it may be wise to do several runs or to compare your results with those of other groups doing the same experiment.

Don't forget to record your inputs on one of the computer INPUT SHEETS (included at the back of this Manual) before you begin.

When you have completed Experiment 3, you should be able to answer the following question:

WHAT GENERAL PATTERN OF ACTIVITY CAN BE EXPECTED FOR AN ENZYME WITH TWO IONIZED AMINO ACIDS AT ITS ACTIVE SITE, IF ONE IS ACIDIC AND THE OTHER BASIC?

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #1*

1) At what pH values (high or low) are the acidic amino acids charged?

2) At what pH values (high or low) are the basic amino acids charged?

3) Pepsin works best only at low pH values of 1 or 2. What type(s) of charged amino acids might be found at its active site?

4) Most enzymes in living cells work best around a pH of 6, 7 or 8 and do not work at all in very high or very low pH's. What kind(s) of amino acids might cause this behavior?

[illegible] #2 [illegible] ENZYME ACTIVITY OF THE NUMBER OF [illegible] AMINO ACIDS AT THE ACTIVE SITE.

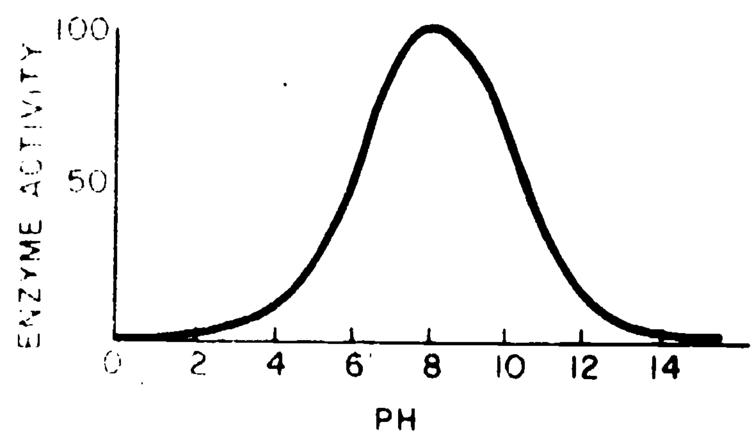
[illegible] noticed in *INVESTIGATION #1*, an increase in the number of [illegible] often restricts the range of pH in which the enzyme will function. [illegible] is often desirable; for instance, we want pepsin to function only in [illegible] strongly acidic stomach and not in the slightly alkaline intestine.

[illegible] the question that if two amino acids at the active site (one basic and the other acidic) can produce a restricted activity range, why [illegible] have more than two ionizable amino acids at its active site? [illegible] answer to this question lies in the possibility of the ionizable amino acids doing more than just restricting the pH range over which the enzyme functions; they may also be taking part in the catalysis of the substrate. Therefore, if an enzyme required more than two types of ionizable amino acids to carry out catalysis, there would be more than two present.

[illegible] investigation is designed to allow you to explore the consequences of adding one or two more charged amino acids at the active site. [illegible] follow the outline of experiments offered here, or ([illegible] allows) experiment on your own.

[illegible] IS THERE MORE THAN ONE COMBINATION OF TWO AMINO ACIDS THAT WILL PRODUCE A MAXIMUM ACTIVITY IN THE RANGE OF pH 7?

[illegible] different combinations of two amino acids (they need not be [illegible]), you should be able to find a combination that produces a pattern of [illegible] activity at pH 7 with a sharp falling off of [illegible] side (see sample graph below).



[illegible] easier to first determine the individual [illegible] the amino acids.)

Experiment 2 WHAT IS THE EFFECT OF ADDING A THIRD AMINO ACID AT THE ACTIVE SITE?

Once you have completed Experiment 1, it is possible to extend that line of inquiry to see the effect of adding a third ionizable amino acid at the active site. What is the effect of adding an amino acid already present as opposed to an amino acid not currently at the active site?

Question: The addition of which amino acid had the least effect on activity?

Experiment 3 WHAT IS THE EFFECT OF ADDING A FOURTH AMINO ACID AT THE ACTIVE SITE, ASSUMING THAT ACTIVITY IS MODERATE TO HIGH WITH THREE IONIZABLE AMINO ACIDS PRESENT?

Select the combination from Experiment 2 that resulted in the highest relative activity at pH 7. Find the effect of adding another amino acid at the active site.

Question: If enzyme activity is high with three ioni[illegible]able amino acids present, will the addition of a fourth amino acid improve the relative activity of the enzyme?

(Optional) Is there another combination of amino acids that will produce higher activity at pH 7?

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #2*

1) All other factors being the same, does the addition of another ionizable amino acid improve the activity of an enzyme?

2) Why might a certain enzyme have three or four ionizable amino acids at its active site?

3) While knowledge of the detailed structure of most enzymes is still lacking, there are indications that most enzymes have only one or two ionizable amino acids at the active site. Why might this make sense from the investigation that you just carried out?

4) Assume that an enzyme works well with two ionizable amino acids at the active site. If an animal were born with a mutation that caused a third ionizable amino acid to be placed at the active site, what would be the chances that the mutation would be passed on? WHY?

INVESTIGATION #4 INVESTIGATION OF THE ACTIVE SITE OF THE ENZYME ACETYLCHOLINESTERASE

The enzyme acetylcholinesterase catalyzes the breakdown of the chemical acetylcholine. This reaction is very important for the proper functioning of the nervous system.

First you'll be given some necessary background on this enzyme and its active site, then you'll be asked to hypothesize which ionizable amino acids might be located at the active site. This question you can attempt to answer using the PH program. The method of investigation is totally up to you.

Background - Acetylcholinesterase catalyzes the following reaction in living systems:

Acetylcholine ⟶ Acetic Acid + Choline

pH studies of this enzyme show it to have a maximum activity at approximately 8.5. When its activity is plotted against pH, a "bell-shaped curve" results.

Studies of both the enzyme and the substrate indicate the active site may look like the following:

It is thought that there are two ionizable amino acids at the active site. The amino acid at site A in the diagram above is thought to carry a negative (−) charge while the amino acid at site B is thought to remain uncharged when the enzyme is active.

Your task is to determine which amino acid(s) might be at sites A and B.

NOTE: Only the amino acid at site A is charged. (The amino acid at site B could be charged initially, but for the period of time the enzyme is active it does not carry a charge.)

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #3*

1) Should the amino acid at site A be acidic or basic? WHY?

2) If there were no ionizable amino acid at site B, what would the sketch of the activity curve look like?

3) If there were an uncharged ionizable amino acid at site B, should it be acidic or basic? WHY?

4) What amino acid(s) is probably at site A?

5) What amino acid(s) is probably at site B?

6) (Optional) Are there any other combinations of amino acids (charged or uncharged) that might produce maximum activity at or near pH 8.5?

Name ____________

PH COMPUTER INPUT SHEET

INVESTIGATION #____ Experiment #____

	RUN 1	RUN 2	RUN 3	RUN 4
NO. IONIZABLE AMINO ACIDS	____	____	____	____
AMINO ACID 1 CODE NO.	____	____	____	____
CHARGED (1=YES, Ø=NO)	____	____	____	____
AMINO ACID 2 CODE NO.*	____	____	____	____
CHARGED (1=YES, Ø=NO)	____	____	____	____
AMINO ACID 3 CODE NO.*	____	____	____	____
CHARGED (1=YES, Ø=NO)	____	____	____	____
AMINO ACID 4 CODE NO.*	____	____	____	____
CHARGED (1=YES, Ø=NO)	____	____	____	____
PH RANGE:				
LOWER LIMIT	____	____	____	____
UPPER LIMIT	____	____	____	____

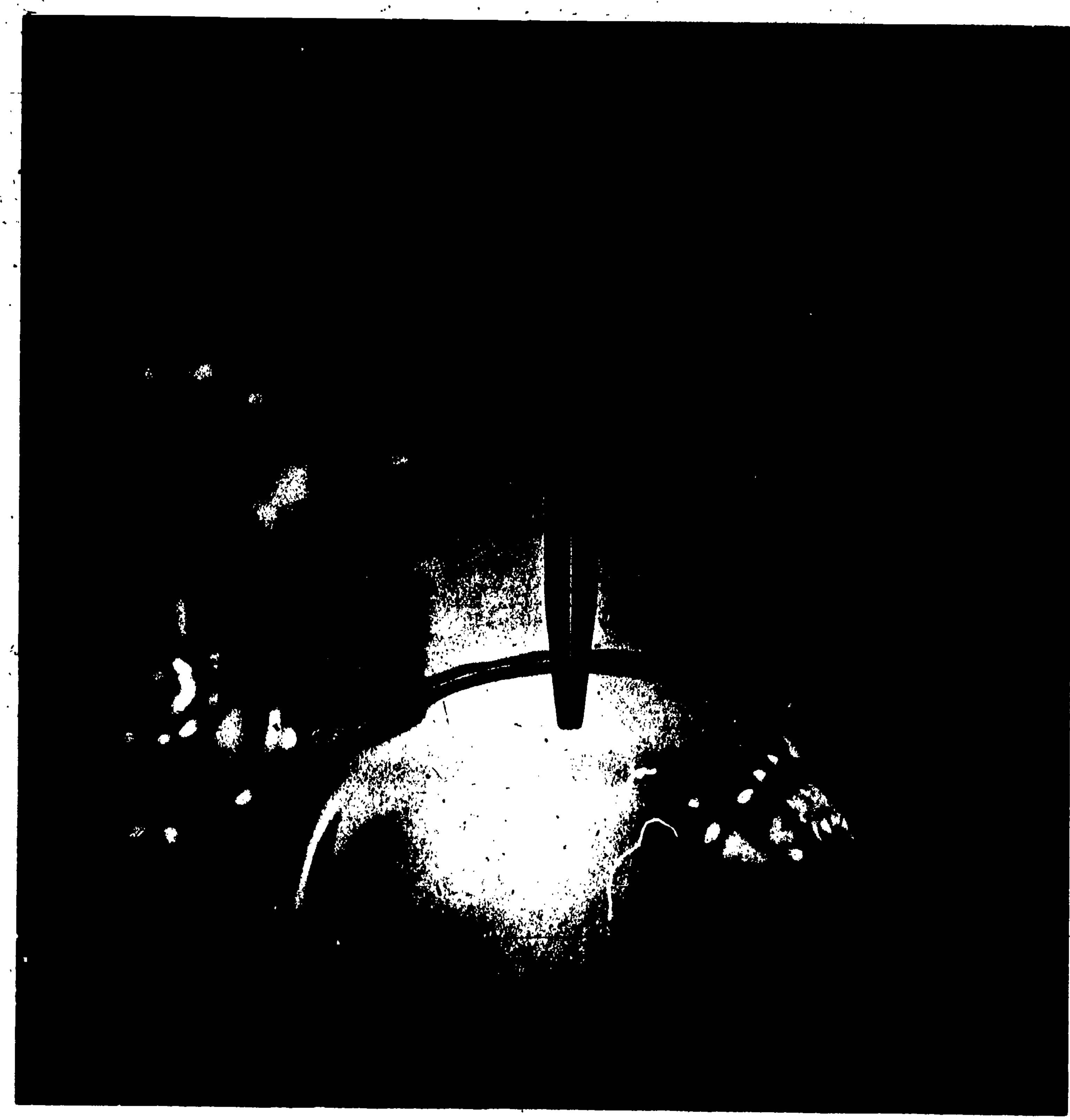
*If you are using fewer than 4 ionizable amino acids, leave an appropriate number of these blank.

DIGITAL EQUIPMENT CORPORATION

TEACHER'S GUIDE $ .50

education

Huntington II Simulation Program - PH



digital

DIGITAL EQUIPMENT CORPORATION
EDUCATIONAL PUBLICATIONS

A partial list of the publications in the continuing series of curriculum material published by Digital for use with EduSystems and RSTS are listed below. Please inquire directly for prices on classroom quantities.

These publications may be ordered (cash enclosed) from:

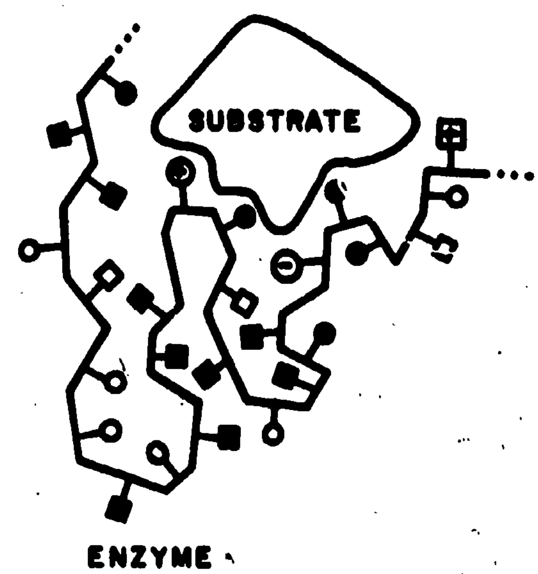
Software Distribution Center
Digital Equipment Corporation
Maynard, Massachusetts 01754

Population: Self Teaching BASIC Workbook		$2.00
101 BASIC Computer Games		$5.00
Understanding Mathematics and Logic Using BASIC Computer Games		$1.50
BASIC Matrix Operations, Project Solo		$1.00
Computer Augmented Calculus Topics		$1.50
Problems for Computer Mathematics		$1.25
Advanced Problems for Computer Mathematics		$2.00
Tutorial Exercises for Chemistry	Student Workbook	$1.00
	Teacher's Resource	$2.75
BASIC Application Programs		
Mathematics I		$1.00
Mathematics II		1.00
Science		1.00
Business and Social Studies		1.00
Plotting		1.00
Chemistry		1.50
Huntington I Application Programs		
Mathematics		$2.00
Teacher Assistance		1.00
Huntington I Simulation Programs		
Biology		$1.00
Chemistry		2.00
Earth Science		1.00
Physics		2.00
Social Studies		1.00
Huntington II Simulation Modules		
Student Workbook		$.30 - .50
Teacher's Guide		.30 - .50
Resource Handbook		.50 - 1.00
Program Paper Tape		.50 - 1.00

TEACHER MANUAL

PH

THE PH SPECIFICITY OF ENZYMES



Developed by:

James Friedland, General D. MacArthur High School
Levittown, New York

Support Material by:

James Friedland
Kirsten Moy, State University of New York
Stony Brook, New York

Programmed by:

James Friedland
Larry Kaufman, State University of New York
Stony Brook, New York

HUNTINGTON TWO COMPUTER PROJECT

14 October 1973

The work of the Huntington Two Computer Project is partially supported by the National Science Foundation, Grant GW-5883.

PUBLISHED EXCLUSIVELY BY DIGITAL EQUIPMENT CORPORATION, 1973

PH

TEACHER MANUAL

Table of Contents

TEACHER MANUAL

I. BASIC INFORMATION ABOUT THE UNIT

Subject Area: Biology

Special Topic: Enzyme Studies

Grade Level: 10th - 12th

Computer Language: BASIC

Special Language Feature: TAB

Abstract: The *PH* program consists of three different laboratory investigations dealing with the pH specificity of enzymes. The purpose of the program is to enable students to determine a possible explanation for pH specificity in an experimental, yet now mathematical, fashion.

II. INTRODUCTION

The program *PH** and its related STUDENT MANUAL are not designed to replace the many worthwhile student experiments that can be carried out in the area of enzyme pH specificity (e.g., BSCS Yellow's Investigation 6-1). Rather, *PH* is designed to allow the student to investigate a possible "why" for pH specificity.

This area of biology is highly mathematical. Since the mathematics is generally beyond the tenth grade study of biology, the explanation of a biochemical phenomenon must often be taken on faith. The *PH* program was designed to present an explanation of the pH specificity of enzymes in a way that would allow students to sidestep the mathematics involved.

The *PH* materials outline three different laboratory investigations. You will probably not want to carry out all of these experiments, but we wanted to offer interested students further activities in this field. The first investigation deals with why many enzymes exhibit a bell-shaped curve when their activity is plotted against different pH values. The second exercise provides an introduction into the importance of structure in an enzyme. The third and last investigation deals with a specific enzyme, *acetylcholinesterase*. This enzyme was the study model for another HUNTINGTON TWO program, *LOCKEY*, and teachers looking for continuity may want to use this exercise.

---

*We will use the convention of capital *PH* when referring to the program and materials and the conventional pH when referring to hydrogen ion concentration.

III. SAMPLE RUN

```
DO YOU WISH INSTRUCTIONS (1=YES, 2=NO)? 1

  CURRENT THEORY HOLDS THAT THE BEHAVIOR OF ENZYMES IS IN PART
A FUNCTION OF THE TYPES OF AMINO ACIDS AT THE ACTIVE SITE.
THIS PROGRAM ALLOWS YOU TO SET THE NUMBER AND TYPE OF
IONIZABLE AMINO ACID(S) PRESENT.

  THE COMPUTER WILL FIRST ASK:
'HOW MANY IONIZABLE AMINO ACIDS AT THE ACTIVE SITE?'
YOU CAN PICK ANY WHOLE NUMBER FROM 1 TO 4.  REAL ENZYMES
ARE THOUGHT TO HAVE AT LEAST TWO AMINO ACIDS
PRESENT, BUT TRY DIFFERENT NUMBERS.

  THEN THE COMPUTER WILL ASK:
'AMINO ACID 1 ---- CODE NO.?'
YOU CAN INPUT THE CODE NUMBER FOR ANY OF THE FOLLOWING AMINO ACIDS:
  CODE     NAME            R-GROUP OF AMINO ACID
  ----     ----            ---------------------
   1    ASPARTIC ACID      -CH2-COOH
   2    CYSTEINE           -CH2-SH
   3    GLUTAMIC ACID      -CH2-CH2-COOH
   4    TYROSINE           -CH2-C6H4-OH
                                             NH2
                                              |
   5    ARGININE           -CH2-CH2-CH2-NH-C=NH
   6    HISTIDINE          -CH2-C3N2H3
   7    LYSINE             -CH2-CH2-CH2-CH2-NH2

AMINO ACIDS 1-4 ARE CALLED ACIDIC AMINO ACIDS
AMINO ACIDS 5-7 ARE BASIC
BY TRYING ACIDIC AND BASIC AMINO ACIDS SEPARATELY AND IN
COMBINATION YOU CAN DEVELOP TYPICAL PH PATTERNS FOR ENZYMES.
```

```
HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)? 2

AMINO ACID 1  ---- CODE NO.? 4
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1
AMINO ACID 2  ---- CODE NO.? 7
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1

PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT? 7
  UPPER LIMIT? 13

                RELATIVE ENZYME ACTIVITY
           0    10   20   30   40   50   60   70   80   90   100
  PH       I----I----I----I----I----I----I----I----I----I----I
  7       I*
          I*
  8       I*
          I *
  9       I    *
          I       *
  10      I          *
          I        *
  11      I    *
          I *
  12      I*
          I*
  13      I*
```

IV. INSTRUCTIONS FOR RUNNING *PH*

The *PH* program requires inputs to the following questions:

1) DO YOU WISH INSTRUCTIONS (1=YES, Ø=NO)?

   If the students have studied this material, instructions would be time-consuming and unnecessary. Many teachers may wish to delete lines 19Ø - 24Ø and lines 3ØØ - 43Ø to eliminate the instructions.

2) CODE LIST (1=YES, Ø=NO)?

   While the code list is handy, it too is duplicated in the *STUDENT MANUAL*. Those teachers wishing to eliminate both instructions and the code list can delete lines 19Ø - 6ØØ.

3) HOW MANY IONIZABLE AMINO ACIDS AT THE ACTIVE SITE (UP TO 4)?

   For *INVESTIGATION #1* the student will choose either 1 or 2. In general, the more amino acids chosen, the more restricted will be the resulting pH range of the enzyme chosen.

4) AMINO ACID 1 ---- CODE NO.?

   The input here is a number corresponding to a particular amino acid. Each amino acid and its corresponding number can be found on the code list.

   (*NOTE:* The order of the code list is semi-alphabetical, with all acidic amino acids listed first. Order does not denote "strength" or any other chemical property.)

5) SHOULD AMINO ACID BE CHARGED (1=YES, Ø=NO)?

   This question is the most difficult for students to understand. It means: "Should the amino acid just named by code be in a charged condition if the enzyme is to have catalytic activity?" For *INVESTIGATIONS #1* and *#2* the answer to this question is always assumed to be "yes." This question is, at least at this point, both for *INVESTIGATION #3* and for the interested student who may want to investigate the *PH* model further.

   (*NOTE:* If the answer to Question #3 was 2 or more, the program will jump back to Question #4 until all amino acid information is inputted.)

6) PH RANGE FOR THIS EXPERIMENT

   LOWER LIMIT?
   UPPER LIMIT?

   When the student has an idea as to when a change in activity will occur, he can shorten the output by narrowing the pH range for the experiment. If your students are just beginning, it is best to encourage them to use 0 to 14 (or at least 2 to 12).

Graphical Output

Since the *PH* model is based on many approximations, a graphical output is both more honest and more comprehensible. You may want to have your students connect the points of the graph to improve visibility, especially if the whole class will be observing these results.

V. USING *PH* IN THE CLASSROOM

Most biology students are taught that enzymes operate best only at certain pH levels, and this knowledge is essential for understanding certain phenomena such as the functioning of the human digestive tract. It is very difficult to teach why most enzymes show a pH optimum, however, without introducing mathematics too complex for most tenth graders. *PH* was designed in an attempt to answer this question without introducing the underlying mathematics.

A. Preparation

The following concepts are important for a student's understanding of the *PH* program:

- ▷ protein structure
- ▷ the nature of a catalyst
- ▷ the idea that amino acids can pick up a positive (+) or negative (-) charge (acidic and basic amino acids).
- ▷ the active site of an enzyme (see p. 5, *RESOURCE MANUAL*).

Materials you should have on hand while using *PH*:

- ▷ Copies of pp.1-3 of the *STUDENT MANUAL*, with names, structures and codes for the acidic and basic amino acids.
- ▷ Tape or tacks to hang up graphical output after each run. (If you use a fresh ribbon in the teletype, most students should be able to see the pattern from their seats.)

B. Hints for Using *PH*

1) As a classroom tool

a) Run *INVESTIGATION #1*, as described in the *STUDENT MANUAL*, before class. *PH* allows combinations of amino acids that probably would not function in nature. These combinations will yield poor results.

b) Teletypes are fun. Allow the student asking the "What if?" question to input the proper information.

c) Since complete graphical output may take a few minutes on some systems, have students hypothesize about the results, under the assumption that the enzyme is active only when all the amino acids are ionized: "What shape will the activity graph have?" or "Will the activity be greater or lower at pH 7?", etc.

d) Bring up the example of *leucine amincpeptidase* (see p.6, RESOURCE MANUAL), an enzyme that doesn't have a single pH optimum, and have the students explain why this enzyme doesn't show a bell-shaped curve when its activity is plotted against different pH values.

2) As a laboratory

Many teachers have found it easiest to use computer-oriented exercises with part of the class while carrying out a related laboratory for the rest of the class. This doubles the exposure the individual student can have, especially if you have access to only a single teletype. In addition, this approach tends to make the students rely more on themselves and the *STUDENT MANUAL*, as you may be busy with those students carrying out the alternative laboratory. Some suggestions for alternative labs that would work well with *PH* include the BSCS Yellow Investigation 6-1, BSCS Blue Investigation 6-4, or S-15. Most other lab books also contain enzyme investigations.

If this approach is not possible, you can still use *PH*, since students should be able to learn by observing the results of different runs.

INVESTIGATION #1 - This investigation develops most of the key concepts, including the nature of enzyme patterns, the role of amino acids at the active site, and the importance of the number and types of amino acids present.

There are three parts to this investigation. If all three parts must be carried out in the same laboratory session, it would be advisable to have students break up into groups of 2 or 3, with 1/4 of the groups assigned to Experiment 1, 1/4 assigned to Experiment 2, and the rest of the group assigned to Experiment 3. By careful organization of groups, it should be possible to cover every possibility within a double-period laboratory session (or over two days).

When each group of students has finished at the computer teletype and made observations on the outputted data, have them hang up their output, carefully noting the number and types of amino acids used, so that other groups might also observe the results. This technique should enable students to arrive at the answers to all the follow-up questions for the investigation.

INVESTIGATIONS #2 and #3 - These two experiments require either a great deal of trial-and-error work or previous experience with *INVESTIGATION #1*; therefore, it is not advisable to attempt either #2 or #3 with whole class groups. These investigations have been included in the *STUDENT MANUAL* (rather than in the *RESOURCE MANUAL*) for the benefit of interested students or groups of students who are working on their own.

3) With individuals or small groups

If you have computer facilities available to you over long periods of time, an individualized approach to *PH* will give the student maximum investigative experience. *PH* has been designed so that there is seldom a single correct solution; an organized experimental approach, however, will usually give the best results in a minimum of time. If time is not a major problem, we recommend your students work through all three investigations.

As far as background material is concerned, you may want to provide those students working on their own with appropriate readings rather than lecturing to them; the *RESOURCE MANUAL* is keyed for this purpose. The information contained in the background readings in addition to the basic material in a standard text should be sufficient for these students to run *PH*.

VI. KEY FOR STUDENT INVESTIGATIONS AND FOLLOW-UP QUESTIONS

INVESTIGATION #1 PATTERNS OF ENZYME ACTIVITY

Experiment 1 WHAT PATTERN OF ENZYME ACTIVITY WOULD BE EXPECTED IF AN ENZYME HAD ONLY ONE CHARGED ACIDIC AMINO ACID AT ITS ACTIVE SITE?

Correct Inputs (You may wish to check the student's INPUT SHEET either before or after carrying out this laboratory)

Correct Inputs

NO. OF IONIZABLE AMINO ACIDS? 1

AMINO ACID 1 ---- CODE NO.? 1,2,3 or 4

SHOULD IT BE CHARGED (1=YES, Ø=NO)? 1

PH RANGE FOR THIS EXPERIMENT

LOWER LIMIT? Ø

UPPER LIMIT? 14

(Other numbers may be used here; for example, to save time, 2 to 12 might be used.)

Sample Output - For amino acid #1 *(aspartic acid)* assuming charged condition necessary for enzyme activity:

```
          RELATIVE ENZYME ACTIVITY
     0    10   20   30   40   50   60   70   80   90   100
PH   I----I----I----I----I----I----I----I----I----I----I
0   I*
    I*
1   I*
    I*
2   I*
    I*
3   I *
    I    *
4   I          *
    I                      *
5   I                                    *
    I                                            *
6   I                                                *
    I                                                 *
7   I                                                  *
    I                                                  *
8   I                                                  *
    I                                                  *
9   I                                                  *
    I                                                  *
10  I                                                  *
    I                                                  *
11  I                                                  *
    I                                                  *
12  I                                                  *
    I                                                  *
13  I                                                  *
    I                                                  *
14  I                                                  *
```

Other Possible Solutions for Experiment 1

An enzyme containing:

amino acid #2 (*cysteine*) will show low activity in a pH range of 0-7, and high activity in a pH range of 9-14;

amino acid #3 (*glutamic acid*) will show low activity in the range 0-3, and high activity in the range 5-14;

amino acid #4 (*tyrosine*) will show low activity in the range 0-9, and high activity in the range 11-14.

Student Question for Experiment 1

IN WHICH PH RANGE, 0-7 or 7-14, WOULD AN ENZYME THAT REQUIRES ONE CHARGED ACIDIC AMINO ACID WORK BEST?

Answer: The specific pH's at which the enzyme might operate best depends on the nature of the amino acid at the active site. All acidic amino acids tend to be ionized at high pH values, however, so that if the enzyme requires this ionization for activity, it would operate best in the range 7 to 14.

Additional Run for Experiment 1

```
HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)? 1

AMINO ACID 1   ---- CODE NO.? 4
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1

PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT? 7
  UPPER LIMIT? 13

           RELATIVE ENZYME ACTIVITY
      0    10   20   30   40   50   60   70   80   90   100
 PH   I----I----I----I----I----I----I----I----I----I----I
 7   I*
     I*
 8   I*
     I *
 9   I   *
     I           *
 10  I                       *
     I                                    *
 11  I                                             *
     I                                                *
 12  I                                                 *
     I                                                  *
 13  I                                                  *
```

29

INVESTIGATION #1

Experiment 2 — WHAT ENZYME ACTIVITY PATTERN WOULD BE EXPECTED FOR AN ENZYME THAT REQUIRED ONE CHARGED BASIC AMINO ACID AT THE ACTIVE SITE FOR ENZYME ACTIVITY?

NOTE: Explanation of the computer inputs are in the *STUDENT MANUAL* only under Experiment 1. If the students have not carried out Experiment 1, they should read the *STUDENT MANUAL* for that experiment.

	Correct Inputs
NO. OF IONIZABLE AMINO ACIDS?	1
AMINO ACID 1 ---- CODE NO.?	5, 6 or 7
SHOULD IT BE CHARGED (1=YES,Ø=NO)?	1
PH RANGE FOR THIS EXPERIMENT	
LOWER LIMIT?	Ø
UPPER LIMIT?	14

Sample Output - For amino acid #6 (*histidine*), assuming ionized condition necessary for enzyme activity:

```
          RELATIVE ENZYME ACTIVITY
       0   10   20   30   40   50   60   70   80   90  100
PH     I----I----I----I----I----I----I----I----I----I----I
 0    I                                                  *
      I                                                  *
 1    I                                                  *
      I                                                  *
 2    I                                                  *
      I                                                  *
 3    I                                                  *
      I                                                  *
 4    I                                                  *
      I                                                  *
 5    I                                                 *
      I                                                *
 6    I                                           *
      I                                 *
 7    I                   *
      I        *
 8    I   *
      I *
 9    I *
      I *
10    I *
      I *
11    I *
      I *
12    I *
      I *
13    I *
      I *
14    I *
```

Other Possible Solutions for Experiment 2

An enzyme containing:

amino acid #5 (*arginine*) at its active site will show low activity in a pH range of 3-14 and high activity in range 0-2;

amino acid #7 (*lysine*) at its active site will show low activity in the range 11-14 and high activity in range 0-9.

Student Question for Experiment 2

OVER WHICH PH RANGE, 0-7 or 7-14, SHOULD AN ENZYME THAT REQUIRES ONE CHARGED BASIC AMINO ACID WORK BEST?

<u>Answer</u>: Just as with acidic amino acids at the active site, there is variation in behavior from amino acid to amino acid; but we should expect such an enzyme to be active at pH values 0 - 7.

Additional Run for Experiment 2

```
HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)? 1

AMINO ACID 1  ---- CODE NO.? 7
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1

PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT? 7.5
  UPPER LIMIT? 12.5

            RELATIVE ENZYME ACTIVITY
     0    10   20   30   40   50   60   70   80   90   100
PH   I----I----I----I----I----I----I----I----I----I----I
     I                                                 *
8    I                                                 *
     I                                               *
9    I                                            *
     I                                     *
10   I                        *
     I           *
11   I    *
     I  *
12   I*
     I*
```

INVESTIGATION #1

Experiment 3 WHAT PATTERN OF ENZYME ACTIVITY WOULD BE ASSOCIATED WITH AN ENZYME THAT REQUIRES TWO CHARTED AMINO ACIDS AT THE ACTIVE SITE, ONE ACIDIC AND ONE BASIC?

NOTE: It is necessary for the students to read Experiment 1 before attempting this experiment.

	Correct Inputs
NO. OF IONIZABLE AMINO ACIDS?	2
AMINO ACID 1 --- CODE NO.?	1, 2, 3 or 4
SHOULD IT BE CHARGED (1=YES, Ø=NO)?	1
PH RANGE FOR THIS EXPERIMENT	
LOWER LIMIT?	Ø
UPPER LIMIT?	14

(See Sample Output on following page.)

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #1*

1) At what pH values (high or low) are the acidic amino acids charged?

   The acidic amino acids are generally charged at high pH values. This is because at high pH values there are few hydrogen ions but many hydroxide ions in solution, causing the hydrogen to be removed from the side groups of these amino acids, leaving them with a net negative charge.

2) At what pH values (high or low) are the basic amino acids charged?

   The basic amino acids are generally charged at low pH values. This is because at low pH values hydrogen ions are in excess, an unbalanced situation which moves toward equilibrium by the addition of hydrogen ions to the basic amino acids, leaving each amino acid with a net positive charge.

3) Pepsin works best only at low pH values of 1 or 2. What type(s) of charged amino acids might be found at its active site?

   Since we are restricting our answer to amino acids carrying a charge, there would have to be a basic amino acid at the active site, since at these extremely low pH values only basic amino acids are charged.

4) Most enzymes in living cells work best around a pH of 6, 7 or 8 and do not work at all in very high or very low pH's. What kind(s) of amino acids might cause this behavior?

   There are several correct answers for this question. The most likely response, considering the results of this investigation, is that at least one acidic <u>and</u> one basic amino acid must be present at the active site. Another correct answer to this question would be two acidic amino acids at the active site, one charged and the other uncharged. There are other correct solutions and the best test of an answer would be a run of the *PH* program.

Other Possible Solutions for Experiment 3

There are 12 different solutions for this experiment. In general, they will exhibit bell-shaped curves with various degrees of extent. A few combinations will exhibit no activity.

Student Question for Experiment 3

WHAT GENERAL PATTERN OF ACTIVITY CAN BE EXPECTED FOR AN ENZYME WITH TWO IONIZED AMINO ACIDS AT ITS ACTIVE SITE, IF ONE IS ACIDIC AND THE OTHER BASIC?

Answer: Activity is usually displayed in the middle pH region with little or no activity at the acidic and basic extremes. In general, the curve can be called bell-shaped.

Additional Run for Experiment 3

```
HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)? 2

AMINO ACID 1  ---- CODE NO.? 2
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1
AMINO ACID 2  ---- CODE NO.? 7
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1

PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT? 6
  UPPER LIMIT? 12

               RELATIVE ENZYME ACTIVITY
        0    10   20   30   40   50   60   70   80   90   100
  PH    I----I----I----I----I----I----I----I----I----I----I
  6     I*
        I *
  7     I  *
        I       *
  8     I                 *
        I                              *
  9     I                                      *
        I                                    *
  10    I                         *
        I            *
  11    I     *
        I  *
  12    I*
```

Sample Output - Using amino acids #1 (*aspartic acid*) and #6 (*histidine*) and assuming ionized condition of both necessary for enzyme activity:

```
          RELATIVE ENZYME ACTIVITY
     0    10   20   30   40   50   60   70   80   90   100
PH   I----I----I----I----I----I----I----I----I----I----I
  0 I*
    I*
  1 I*
    I*
  2 I*
    I*
  3 I *
    I    *
  4 I          *
    I                      *
  5 I                                   *
    I                                          *
  6 I                                          *
    I                                 *
  7 I                   *
    I        *
  8 I   *
    I *
  9 I*
    I*
 10 I*
    I*
 11 I*
    I*
 12 I*
    I*
 13 I*
    I*
 14 I*
```

INVESTIGATION #2 *EFFECT ON ENZYME ACTIVITY OF THE NUMBER OF IONIZABLE AMINO ACIDS AT THE ACTIVE SITE.*

There is no single solution to any of the experiments in this investigation. For this reason many teachers will want to avoid this investigation with large groups.

Experiment 1 IS THERE MORE THAN ONE COMBINATION OF TWO AMINO ACIDS THAT WILL PRODUCE A MAXIMUM ACTIVITY IN THE RANGE OF pH 7?

Due to the large number of combinations possible (over 150), there are very likely many amino acid combinations that yield maximum activity in a pH range of 7.

For Experiments 2 and 3, let students work with any of these eligible sets of amino acids. These amino acids may be in the charged or uncharged states.

Experiment 2 WHAT IS THE EFFECT OF ADDING A THIRD AMINO ACID AT THE ACTIVE SITE?

It depends on the type of amino acid added; *usually*, the addition of an amino acid of the same type as one of those already present will have the least effect.

For this experiment, let the students add any of the seven amino acids available, in either the charged or the uncharged state, to satisfy the catalytic requirement.

Experiment 3 WHAT IS THE EFFECT OF ADDING A FOURTH AMINO ACID AT THE ACTIVE SITE OF AN ENZYME, ASSUMING THAT ACTIVITY IS MODERATE TO HIGH WITH THREE IONIZABLE AMINO ACIDS PRESENT?

In general, the greater the number of amino acids, the more restricted the pH range in which an enzyme is active.

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #2*

1) All other factors being the same, does the addition of another ionizable amino acid improve the activity of an enzyme?

> No; in general the addition of another amino acid lowers activity and narrows the range of activity. (See the three sample runs for this experiment.)

2) Why might a certain enzyme have three or four ionizable amino acids at its active site?

> If three or four were required for catalytic activity, they would have to be present or no catalytic activity would result at all. (Note: This is an aspect of *PH* which can lead many students astray. You may wish to emphasize this in your classroom follow-up.)

3) While knowledge of the detailed structure of most enzymes is still lacking, there are indications that most enzymes have only one or two ionizable amino acids at the active site. Why might this make sense from the investigation that you just carried out?

> One of the conclusions the student might come to from this investigation is that the addition of a third or fourth amino acid is disadvantageous, unless there is a good reason for the presence of the extra amino acid(s). (Some geneticists have begun to use this idea as a basis for molecular evolution.)

4) Assume that an enzyme works well with two ionizable amino acids at the active site. If an animal were born with a mutation that caused a third ionizable amino acid to be placed at the active site, what would be the chances that the mutation would be passed on? WHY?

> This is an open discussion question. Some of the above ideas may play an important role in the ultimate decision (or lack of one) by your class.

INVESTIGATION #3 *INVESTIGATION OF THE ACTIVE SITE OF THE ENZYME ACETYLCHOLINESTERASE.*

NOTE: Additional background materials on the enzyme *acetylcholinesterase* may be found in the materials for the HUNTINGTON TWO program *LOCKEY*.

GIVEN (1) THAT THE ENZYME ACETYLCHOLINESTERASE IS KNOWN TO HAVE A pH OPTIMUM IN THE AREA OF 8.5 to 9, AND (2) THAT THERE ARE THOUGHT TO BE TWO IONIZABLE AMINO ACIDS LOCATED AT THE ACTIVE SITE (ONE CHARGED AND ONE UNCHARGED), WHAT TWO AMINO ACIDS ARE RESPONSIBLE FOR THE ENZYME'S pH BEHAVIOR?

Since there are many experimental approaches that might be taken in this investigation, no specific directions have been given. Students might find the additional background material in the *RESOURCE MANUAL* helpful in determining a method of approach.

A Sample Approach to *INVESTIGATION #3*

The following approach is only one of several that should prove successful:

1) First determine the characteristics of each amino acid: when it is ionized, when it is un-ionized, etc.

2) Discard from consideration all amino acids that undergo ionization change in a region other than near pH 9; these amino acids are unlikely to produce an optimum in the proper range, as shown by the results of *INVESTIGATION #1*, Experiment 3.

3) Investigate the combined properties of remaining amino acids, when one amino acid is ionized and the other un-ionized (not charged).

4) Select as the most probable choice the pair that produce an optimum near pH 8.5 to 9 and that meet the requirements noted in the background materials.

5) Also examine other possible combinations that have an optimum around pH 8.5 to 9.

A Possible Solution

Using a mathematical procedure similar to that carried out by the program *PH*, biochemists have come up with the most probable ionizable amino acids at the active site of acetylcholinesterase:

Site A: *cysteine* in the charged condition

Site B: *tyrosine* in the uncharged condition.

The following graph produced by the *PH* program will confirm that the presence of cysteine in a negatively charged state and tyrosine in an uncharged state produces a pH maximum in the proper range and meets the requirements noted in the background materials.

Instructors with a knowledge of catalytic mechanisms may recognize this state as being favorable for acid-base catalysis.

Graphical Output for this Solution

```
HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)? 2

AMINO ACID 1  ---- CODE NO.? 2
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 1
AMINO ACID 2  ---- CODE NO.? 4
 SHOULD IT BE CHARGED (1=YES, 0=NO)? 0

PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT? 0
  UPPER LIMIT? 14

                   RELATIVE ENZYME ACTIVITY
           0    10   20   30   40   50   60   70   80   90   100
 PH        I----I----I----I----I----I----I----I----I----I----I
 0        I*
          I*
 1        I*
          I*
 2        I*
          I*
 3        I*
          I*
 4        I*
          I*
 5        I*
          I*
 6        I*
          I *
 7        I  *
          I       *
 8        I                 *
          I                              *
 9        I                                      *
          I                                     *
 10       I                          *
          I             *
 11       I     *
          I  *
 12       I *
          I*
 13       I*
          I*
 14       I*
```

NOTE: There may be other solutions. Certain biochemists have suggested the use of *histidine*. You may want to examine this possible solution yourself, or have one of your students work with this possibility.

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #3*

1) Should the amino acid at site A be acidic or basic? WHY?

The amino acid at site A should be acidic. In the background materials it was noted that the amino acid at site A was thought to carry a negative charge. Only an acidic amino acid is likely to develop a negative charge through the loss of a hydrogen ion.

(For your information this deduction, in part, came from noting the nature of the substrate. Acetylcholine, the natural substrate, has a positive charge in the equivalent location.)

2) If there were no ionizable amino acid at site B, what would the sketch of the activity curve look like?

In this case the activity plotted against pH would show low activity in the basic pH range, assuming site A contained an acidic amino acid that had to be in the charged state for catalysis.

(Note: If another answer were given for Question #1 above, the response to this question would also be different.)

3) If there were an uncharged ionizable amino acid at site B, would it be acidic or basic? WHY?

If the student has used an ionized (charged) acidic amino acid at site A, then he must use an uncharged acidic amino acid at site B. If a basic amino acid is used, a broad range of pH optimum generally results. (There are some solutions that make use of basic amino acids, however.)

4) What amino acid(s) is probably at site A?

Scientists think cysteine most likely.

5) What amino acid(s) is probably at site B?

Tyrosine seems to be the most likely choice.

6) Are there any other combinations of amino acids (charged or uncharged) that might produce maximum activity at or near pH 8.5?

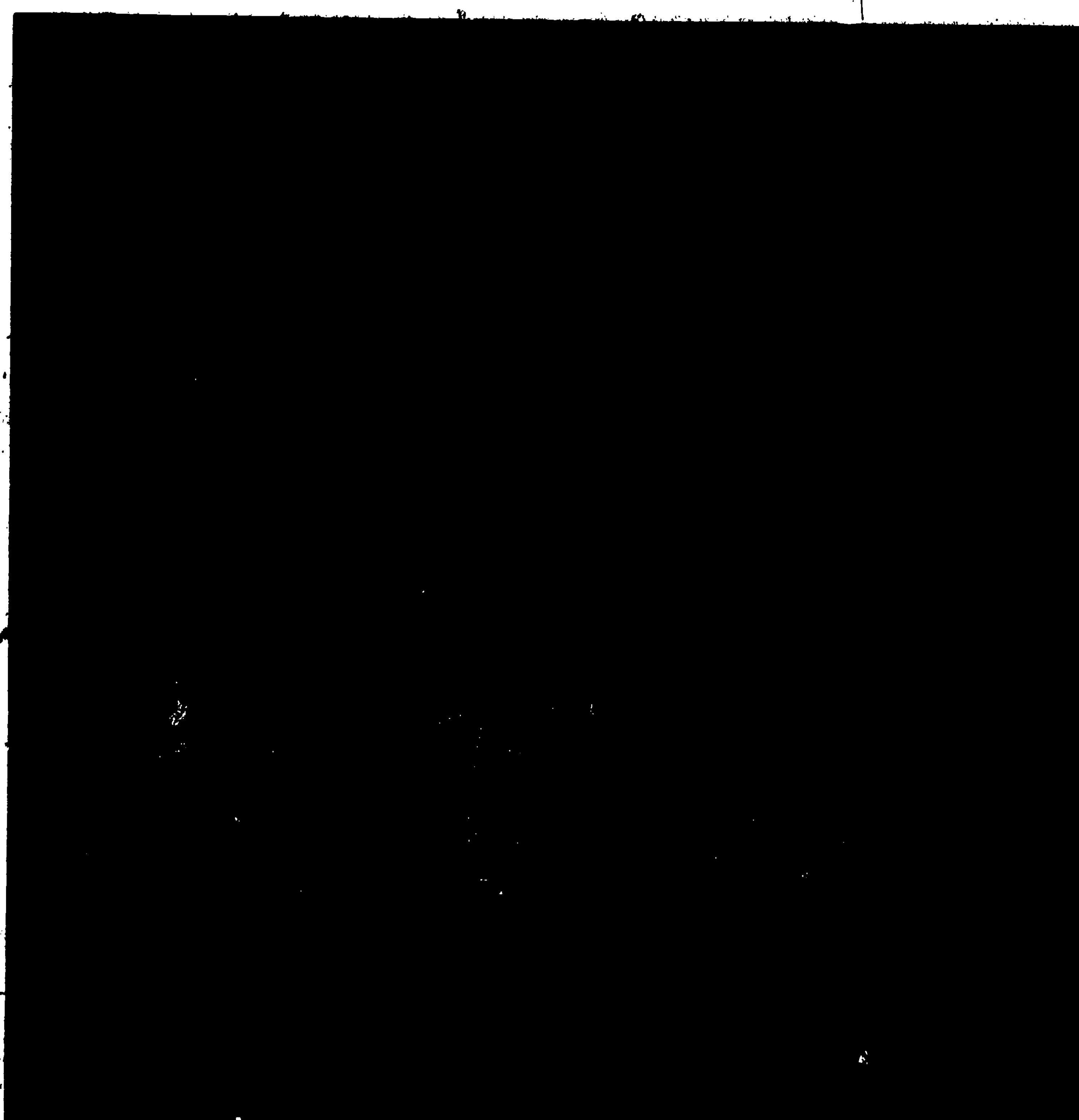
There may well be, since the *PH* program alone allows over 2500 combinations of amino acids.

DIGITAL EQUIPMENT CORPORATION

RESOURCE HANDBOOK $1.00

education

Huntington II Simulation Program - PH



DIGITAL EQUIPMENT CORPORATION
EDUCATIONAL PUBLICATIONS

A partial list of the publications in the continuing series of curriculum material published by Digital for use with EduSystems and RSTS are listed below. Please inquire directly for prices on classroom quantities.

These publications may be ordered (cash enclosed) from:

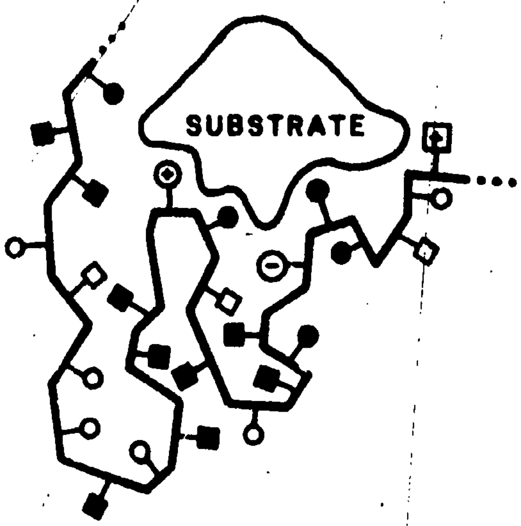
Software Distribution Center
Digital Equipment Corporation
Maynard, Massachusetts 01754

Population: Self Teaching BASIC Workbook		$2.00
101 BASIC Computer Games		$5.00
Understanding Mathematics and Logic Using BASIC Computer Games		$1.50
BASIC Matrix Operations, Project Solo		$1.00
Computer Augmented Calculus Topics		$1.50
Problems for Computer Mathematics		$1.25
Advanced Problems for Computer Mathematics		$2.00
Tutorial Exercises for Chemistry	Student Workbook	$1.00
	Teacher's Resource	$2.75
BASIC Application Programs		
Mathematics I		$1.00
Mathematics II		1.00
Science		1.00
Business and Social Studies		1.00
Plotting		1.00
Chemistry		1.50
Huntington I Application Programs		
Mathematics		$2.00
Teacher Assistance		1.00
Huntington I Simulation Programs		
Biology		$1.00
Chemistry		2.00
Earth Science		1.00
Physics		2.00
Social Studies		1.00
Huntington II Simulation Modules		
Student Workbook		$.30 - .50
Teacher's Guide		.30 - .50
Resource Handbook		.50 - 1.00
Program Paper Tape		.50 - 1.00

RESOURCE MANUAL

PH

THE PH SPECIFICITY OF ENZYMES



Developed by:

James Friedland, General D. MacArthur High School
Levittown, New York

Support Material by:

James Friedland
Kirsten Moy, State University of New York
Stony Brook, New York

Programmed by:

James Friedland
Larry Kaufman, State University of New York
Stony Brook, New York

HUNTINGTON TWO COMPUTER PROJECT

14 October 1973

The work of the Huntington Two Computer Project is partially supported by the National Science Foundation, Grant GW-5883.

PUBLISHED EXCLUSIVELY BY DIGITAL EQUIPMENT CORPORATION, 1973

PH

RESOURCE MANUAL

Table of Contents

RESOURCE MANUAL

INTRODUCTION

Some of the material in this manual is written with the student, rather than the teacher, in mind. It is our hope that an interested student will be able to research and find his own answers to questions posed in the *PH* program, using certain materials in this manual as the necessary background.

Other parts of this manual are clearly meant for the teacher. Those sections that are intended solely for background reading will be marked with a ★, and the sections containing extended materials for the teacher will be marked with a ●. Sections marked with both (★●) indicate a mixed function.

I. BACKGROUND INFORMATION

★ Enzymes as Proteins

Structurally, enzymes are *proteins*; therefore, it is necessary to understand the nature of proteins in order to understand enzymes. Proteins are polymeric. A polymer may be thought of as a group of *similar* units bonded together in a chain. Figure 1 can be used to represent a polymer. In this case, the similar polymer unit is the circle. Notice that not all the circles are exactly the same. The definition of a polymer requires only that the units be similar.

FIGURE 1

We will take the "..." on the end of the chain to mean "and so on." This is another characteristic of most polymers: they are long. Also, the units that make up the polymer are usually bonded together in a particular fashion. We have now stated three requirements of proteins as polymers: they must be made up of similar units in a chain; the chain must be long; and the units should be bonded together in a particular fashion.

The units that make up proteins are called amino acids. All amino acids are structurally similar. Figure 2 below illustrates the basic structure of an amino acid. Each amino acid is based on a central carbon atom (1) to which four different groups are attached: the amino group (2) composed of a nitrogen and two hydrogens, the carboxyl or carboxylic acid group (3), a single hydrogen (4) and a side group or replacement group (5). Of these groups, only the side group is allowed to vary from one amino acid to another.

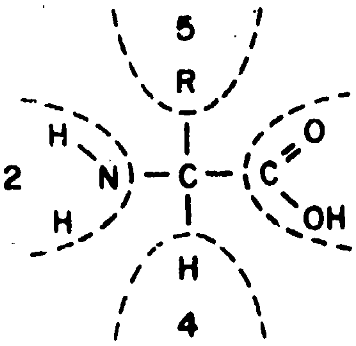
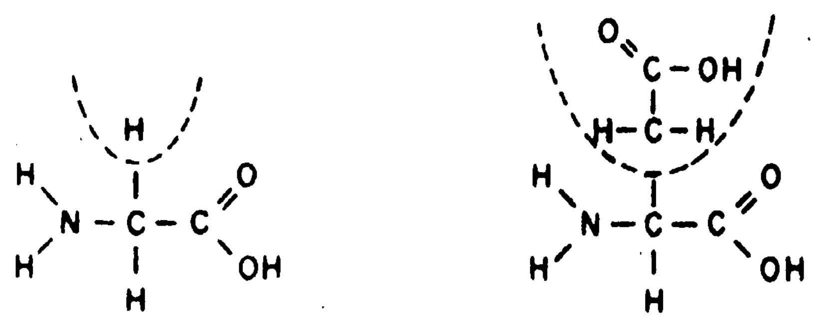


FIGURE 2

In Figure 3 you see two examples of amino acids. In glycine (a) the side group (R) is a single hydrogen atom; in aspartic acid (b) the side group is more complex. Both are amino acids, however, and can be found in protein polymers. There are over 20 other types of amino acids that can be found in proteins. Each of these amino acids (with two exceptions) is structurally identical except for the side (R) group.



(a) *(b)*

FIGURE 3

How long are protein polymers? To get some idea of what "long" means in the case of proteins, let's look at two examples: *insulin* and *collagen*. Insulin is one of the shortest protein polymers known and has 51 amino acids arranged in two chains. Collagen, which is found in your hair and fingernails, can have as many as 3,000 amino acids -- and collagen is nowhere near the largest protein.

Proteins have their amino acid units bonded together by peptide bond . Figure 4 shows the location of a peptide bond. The covalent bond is always between the amino group of one amino acid and the carboxyl group of the other.

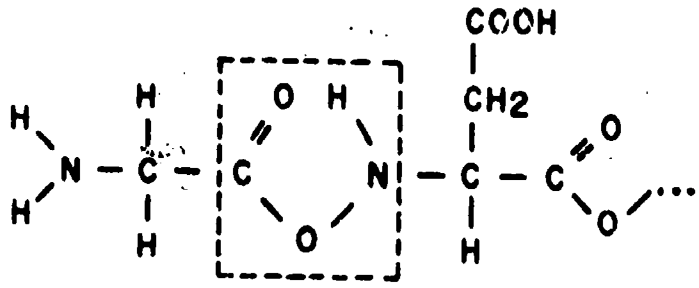


FIGURE 4

The free carboxyl groups can in turn be bonded to the amino group of another amino acid and so on until long chains result.

Proteins are not straight chains; they are folded over and about themselves. Studies have shown that this folding becomes an important consideration when one attempts to determine the properties of a protein. Heat and strong acids and bases are among the many factors that can change the folding of a protein. Think of the change that occurs when you cook egg white. Egg white is a solution of albumin, a protein.

★ Enzymes as Catalysts

We can picture a chemical reaction as a hill. No matter what the grade on the other side of the hill, it is going to take energy to get up to the crest of the hill. If we could in some way lower the top of the hill, with a bulldozer for instance, it would require less energy to get to the other side. Catalysts act by "lowering the hill."

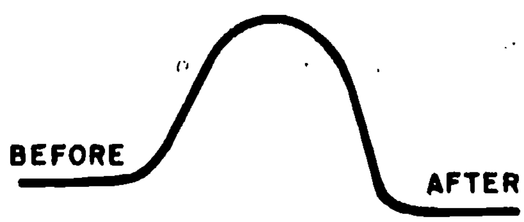


FIGURE 5

For an example, let's take an enzyme called acetylcholinesterase. This enzyme speeds the breakdown of the chemical acetylocholine into two parts, choline and acetic acid.

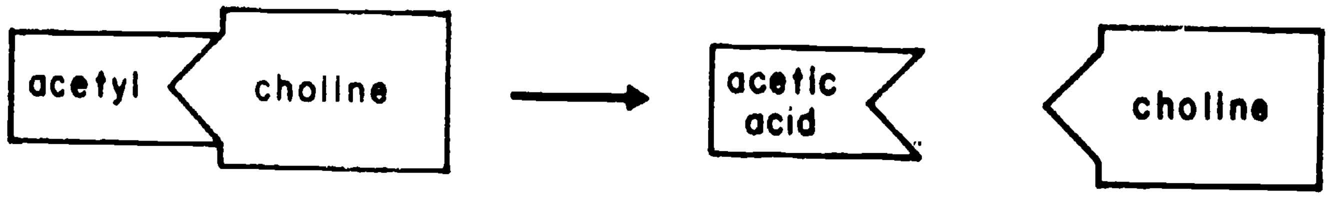


FIGURE 6

This break-up would occur naturally, but at a very slow rate, because the energy required to "get up the hill" is great. (This amount of energy is called the *activation energy* of the reaction.) In fact, the reaction would proceed so slowly that you would die because your nerves would stop functioning. How can we lower the activation energy so that the reaction will proceed more quickly?

To reduce the activation energy, we require a particular enzyme, since each enzyme is <u>specific</u> and is a catalyst for only one of a few reactions. An enzyme cannot lower the activation energy of any of the other reactions that might be going on at the same time. In our case, we require the enzyme acetylcholinesterase.

The enzyme first bonds to the substrate (the substance that the enzyme works on), forming an <u>enzyme-substrate</u>. Most enzymes are thought to have electrically charged groups that in some way lower the amount of energy required for the reaction. When the reaction is over, the enzyme releases the product.

E + S ⟶	E - S ⟶	E - P ⟶	E + P
separate enzyme and substrate	enzyme substrate complex	enzyme and products after reaction	separate enzyme and products after reaction

FIGURE 7

Figure 7 shows the enzyme emerging from the reaction unchanged. This is another characteristic of a catalyst, that it takes part in a reaction but is itself unaltered by the reaction. For this reason, a catalyst (in our case, the enzyme) can be used over and over again. Furthermore, only a small amount of catalyst is required for a reaction involving a large amount of the substrate.

It is possible to depict a reaction in which an enzyme is present as a catalyst in the following way:

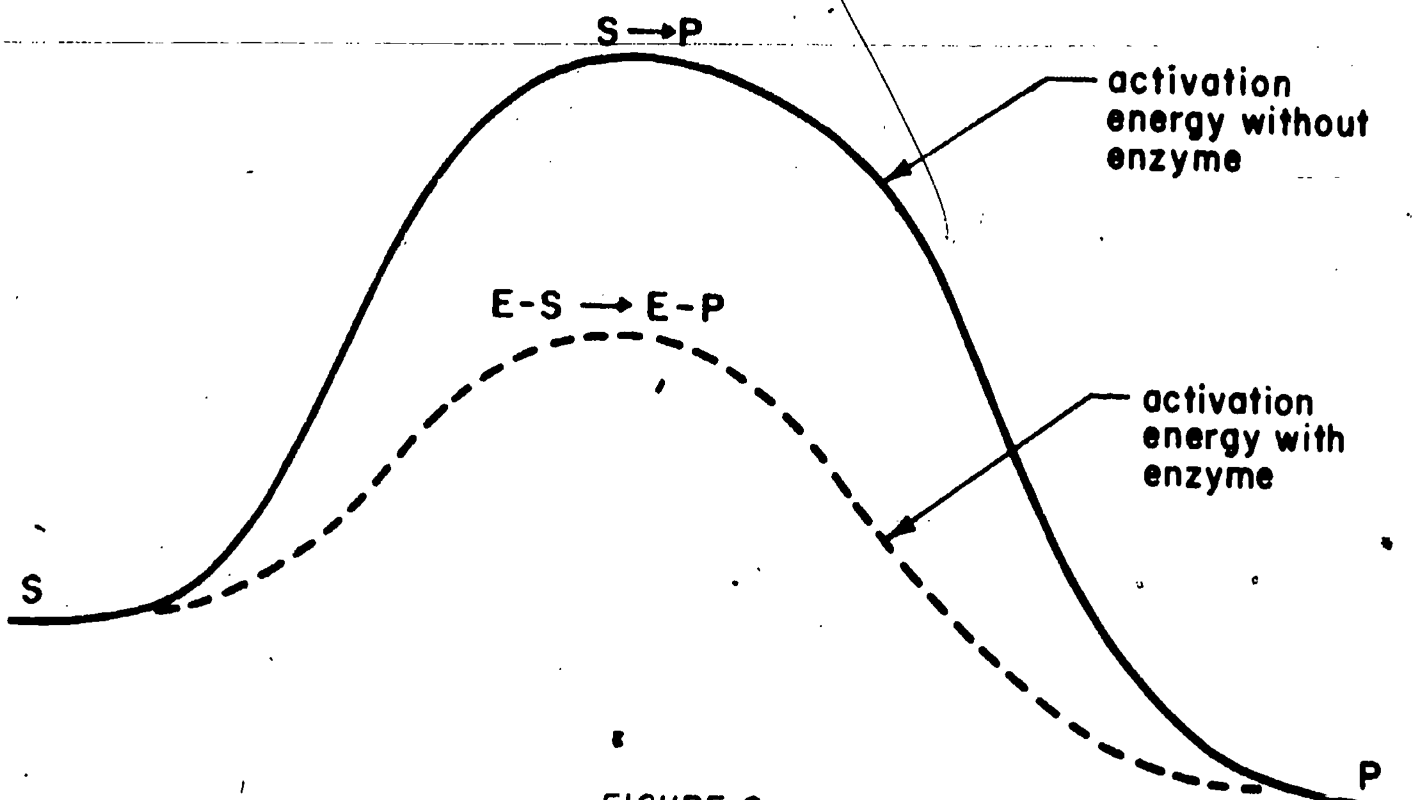


FIGURE 8

The enzyme does not change the substrate or the products that would normally form; it only lowers the activation energy, allowing the reaction to occur more easily and usually more rapidly.

★ Active Site of an Enzyme

Once scientists discovered some of the mechanisms of enzyme action, they tried to answer another question: Does the whole enzyme take part in catalysis? Careful investigations using many different techniques have indicated that certain areas of an enzyme are very important for catalysis, while other areas have no effect on the enzyme's catalytic ability. This leads to the idea of an active site, the area of the enzyme on which catalysis is actually carried out. Many enzymes may have more than one active site, but all the active sites in such cases are involved in the catalysis of the same reaction.

On the pH Specificity of Enzymes

Nearly all enzymes exhibit pH optima. Some specific examples that you may find useful in class discussion are listed below:

Enzyme	pH Optimum
Salivary amylase	5.5 - 6.5
Pepsin	1 - 2
Pancreatic amylase	6.7 - 7.2
Trysin	7.8 - 8.7
Lipase	8 -
Maltase	6.1 - 6.8

The ranges in the above figures reflect the range of experimental determination; but even then the variations are small. Most enzymes have a bell-shaped curve when their activity is plotted against pH (see (a) below).

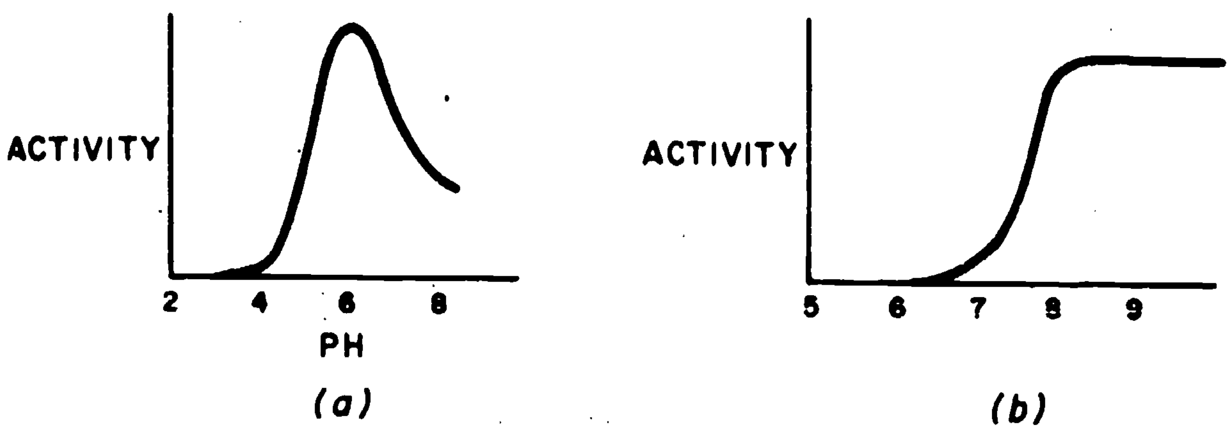


FIGURE 9

However there are a few enzymes such as leucine aminopeptidase that show quite a different curve (see (b)). Why should enzymes in general show a bell-shaped curve and have restricted pH optima? And why do certain enzymes behave differently?

As we said before, the answers to these questions can become highly mathematical; while we do not expect high school students to understand the mathematical solutions involved, we hope that by using *PH* they will acquire a basic understanding of pH specificity -- the norm and the exceptions.

pH can affect (1) the affinity of an enzyme for its substrate; (2) the stability of the enzyme; or (3) the catalytic action of the enzyme. The effects of (1) and (2) can often be screened out of experimental results. When this is done the "bell-shaped curve" still remains. It seems, then, that a pH optimum is due primarily to the effect of different pH values on the catalytic action of the enzyme.

In general enzymes are thought to function through acid-base catalysis; in order for this type of catalysis to occur, there must be charged groups at the active site of the enzyme. Since enzymes are protein structures, the only possible charged groups will be amino acids. Luckily, there are only a few amino acids that can carry a charge on their side groups (R groups).

Those amino acids that can carry a positive charge on their side group (by picking up an additional hydrogen ion) are called basic amino acids. One example of a basic amino acid is lysine:

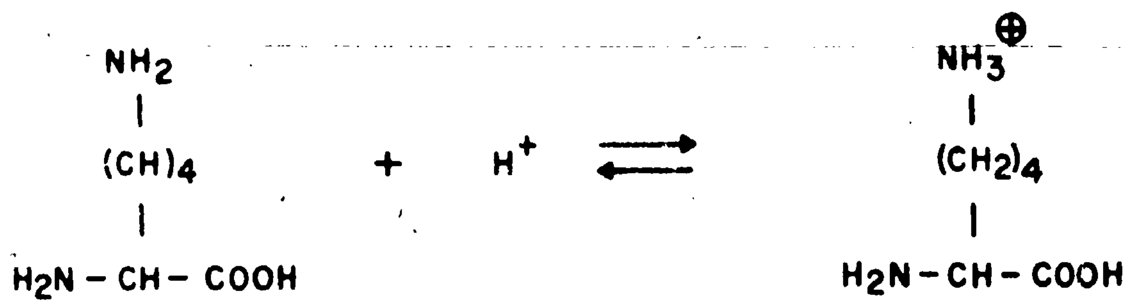


FIGURE 10

Since a hydrogen ion (H^+) is involved in such a reaction, a basic amino acid can carry a net positive charge only at certain pH values. While the pattern is the same for each of the basic amino acids, each basic amino acid has its own range of pH values in which it is charged.

BASIC AMINO ACIDS

pH at which the group is one-half ionized = pK_a

Name	pK_a	R-Group Structure
[illegible]	1.48	$-CH_2-CH_2-CH_2-NH-\overset{NH_2}{\overset{\uparrow}{C}} = NH$
[illegible]	6.8	$-CH_2-C_3N_2H_3$
[illegible]	10.0	$-CH_2-CH_2-CH_2-CH_2-NH_2$

Other amino acids can carry a net negative charge (by releasing a hydrogen ion). These amino acids are called acidic. *Glutamic acid* is one example:

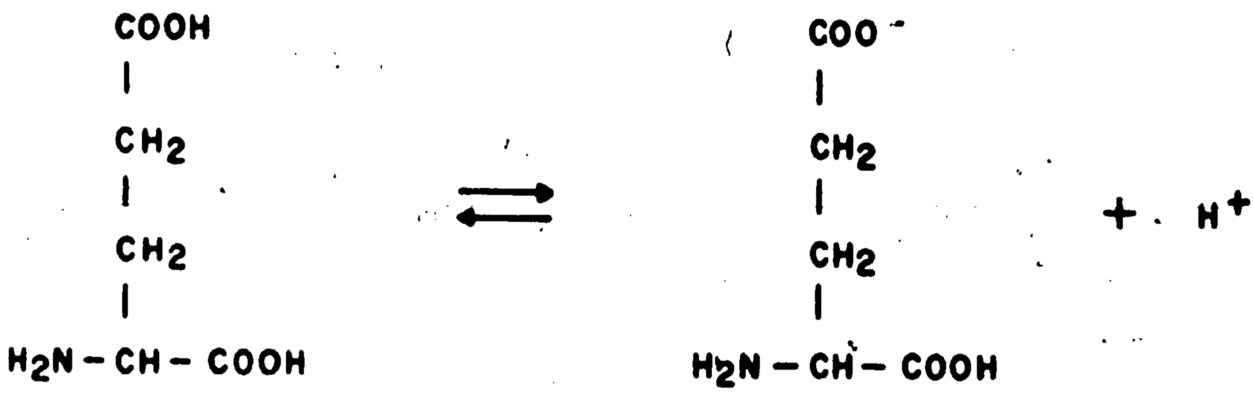


FIGURE II

Likewise, each acidic amino acid has a certain particular range of pH values in which it is charged. For example, *glutamic acid* is highly charged at pH higher than 5, while *tyrosine* is charged only at pH higher than 11.

ACIDIC AMINO ACIDS

Name	pK_a	R-Group Structure
ASPARTIC ACID	4.6	$-CH_2-COOH$
CYSTEINE	8.3	$-CH_2-SH$
GLUTAMIC ACID	4.25	$-CH_2-CH_2-COOH$
TYROSINE	10.7	$-CH_2-C_6H_4-OH$

In general, basic amino acids are charged at low (acidic) pH values, while acidic amino acids are charged at high (basic) pH values. Your students should be able to develop this understanding through experimentation with the program [illegible].

Relation of Enzyme Activity to Amino Acid Charge

If a basic and an acidic amino acid are both present at the active site of an enzyme, a bell-shaped curve may develop. Since the basic amino acid is charged only at low pH values and the acidic amino acid is charged only at high pH values, they both will be charged only in the middle of the pH scale, if at all. If both the acidic and the basic amino acids are required for the enzyme's catalytic activity, then we will find that the enzyme has a restricted pH optimum somewhere around 7 on the pH scale.

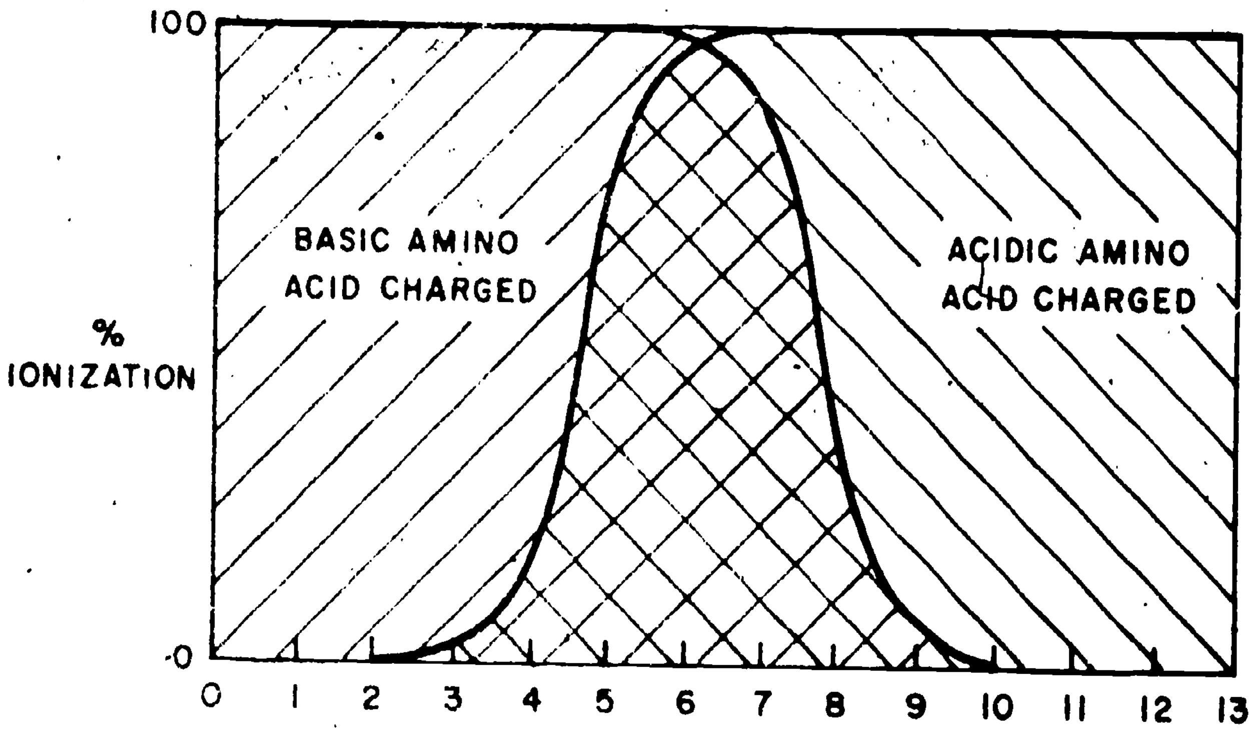


FIGURE 12

This would account for the bell-shaped curves seen for many enzymes. If the presence of both an acidic and a basic amino acid is necessary for the enzyme to function, we should expect to see catalytic activity only when both of them are in a charged state.

How does this explain the activity curve of an enzyme such as leucine aminopeptidase that has a uniform pH optimum over the entire basic range? By looking back at the last graph, you should be able to formulate a hypothesis.

pH and Amino Acid Charge State

As for many other chemical reactions, a change in the charge of an amino acid can be described by an equilibrium equation. For the reaction

$$\text{amino acid} \rightleftharpoons H^+ + \text{amino acid}^-$$

we can write the following equilibrium expression:

$$K_a = \frac{[H^+]\,[\text{amino acid}^-]}{[\text{amino acid}]} \qquad [1]$$

Rearranging terms we obtain:

$$[H^+] = K_a \frac{[\text{amino acid}]}{[\text{amino acid}^-]} \qquad [2]$$

If we assume that only a very small number of the hydrogen ions present are contributed by the water, we can derive an expression for the pH of the solution from Equation [2] above. To do this, first take the log of each side:

$$\log [H^+] = \log K_a + \log \frac{[\text{amino acid}]}{[\text{amino acid}^-]} \qquad [3]$$

Since pH equals the negative log of the hydrogen ion concentration, multiplying Equation [3] through by -1 will give us the desired expression:

$$pH = -\log [H^+] = -\log K_a + \log \frac{[\text{amino acid}^-]}{[\text{amino acid}]} \qquad [4]$$

Note that multiplying through by -1 inverts the fraction. Also, the quantity ($-\log K_a$) is a constant for any single amino acid. This difficult form of the equation can be simplified by introducing a new quantity called the pK_a, the negative log of the constant K_a. By substituting this new quantity into Equation [4] and rearranging terms, we obtain:

$$\log \frac{[\text{amino acid}^-]}{[\text{amino acid}]} = pH - pK_a$$

Using this equation, it is possible to calculate the per cent of amino acid in the charged form at any pH. Although this equation was obtained using an acidic amino acid, a similar equation can be derived for basic amino acids. The program [illegible] evaluates such equations for the student over the entire pH range from 0 to 14.

11. *PH* PROGRAM LISTING

[illegible]

```
1[illegible] LET [illegible]=1
1[illegible] [illegible] [illegible]=1 [illegible]
1[illegible] IF [illegible]([illegible])> [illegible] THEN 1[illegible]
1[illegible] LET L=[illegible]+[illegible](T)
1[illegible] GOTO 1[illegible]
1[illegible] LET L=[illegible](T)-[illegible]
1[illegible] LET [illegible]=1/(1[illegible]L+1)
1[illegible] LET [illegible]=[illegible]
1[illegible] [illegible]
1[illegible] IF [illegible]>[illegible](?) THEN 111[illegible]
11[illegible] [illegible]:
111[illegible] [illegible](5);"I";TAB(INT(5[illegible]+6.5));"[illegible]"
11[illegible] [illegible]
11[illegible] [illegible]
11[illegible] [illegible] "[illegible] (1=[illegible], [illegible]=[illegible])";
115[illegible] [illegible]
116[illegible] IF [illegible]=1 THEN [illegible]1[illegible]
117[illegible] IF [illegible]<>[illegible] THEN 1140
11[illegible] [illegible]
1[illegible] [illegible]
1[illegible] [illegible] "[illegible] IS LIMITED TO FROM 0 TO 1?"
1[illegible] [illegible]
1[illegible] [illegible]
1[illegible] DATA 4.?,?.3,4.?5,1?.?7,-1.4?,-6.?,-1?.?
1[illegible] END
```

III. THE *PH* MODEL

Program Equations and Assumptions

1) Equations derived from the Henderson-Hasselbach equation for weak acids are assumed to be a valid description for the reactions of side groups of amino acids:

$$\log \text{(ionized/de-ionized)} = pH - pK_a \quad \text{for acidic groups}$$

$$\log \text{(ionized/de-ionized)} = pK_a - pH \quad \text{for basic groups}$$

2) When more than one ionizable amino acid is present at the active site, all amino acid side groups are assumed to be equally important in carrying out catalysis:

catalytic ability = (% amino acid #1 with proper charge)
x (% amino acid #2 with proper charge)
x . . . . . .

3) None of the amino acids at the active site are N-terminal or C-terminal; thus alpha amino and alpha carboxyl groups are not significant.

4) Neither the structure of the enzyme nor the structure of the substrate is assumed to have a modifying effect on catalysis.

5) pH is assumed to affect only the enzyme, not the substrate.

6) At no time does the enzyme become irreversibly denatured.

Program Variables and Their Meaning

A(T)	pK_a of amino acid #T multiplied by identifier (+1 for acidic, -1 for basic amino acids)
B	Amino acid code number holder
C	Charged state identifier (-1 means uncharged)
D(T)	pK_a holder
E	Per cent of amino acid in correctly charged state
N	Number of amino acids at active site
P	pH
P1	Lower limit of pH determination
P2	Upper limit of pH determination
Q	Response holder
T	Counter
Z	Catalytic ability of the enzyme

IV. BIBLIOGRAPHY

Sources for Additional Background on *PH*

HIGH SCHOOL TEXTS

[illegible]: AN INQUIRY INTO LIFE
BSCS (Yellow) 2nd Edition
Harcourt, Brace and World, Inc., New York (1968)

Chapter 6, especially pp. 105-109

[illegible]: MOLECULES [illegible] MAN
BSCS (Blue) Revised Edition
Houghton Mifflin Company, Boston (1968)

Chapter 6, especially pp. 142-146

[illegible]
Gregory and Goldman
Ginn and Company, Boston (1971)

Chapter 7, especially pp. 108-109
Chapter 34, especially pp. 537-540

[illegible]: AN INQUIRY INTO THE NATURE OF LIFE
Stanley L. Weinberg
Allyn and Bacon, Inc., Boston (1971)

Chapter 4, especially pp. 76-80

COLLEGE LEVEL GENERAL BIOLOGY TEXTS

Berrill, N. J.
[illegible] IN ACTION
Dodd, Mead and Co., New York (1966)

Chapter 4, especially pp. 48-54

Keeton, William T.
[illegible], 2ND EDITION
W. W. Norton and Company, New York (1972)

pp. 42-44 and pp. 171-175

Phillips, Edwin A.
[illegible]
Macmillan Company, New York (1971)

Chapter 19, especially 192-203

Weisz, Paul B.
[illegible]
McGraw-Hill Book Company, New York (1967)

Chapter 5, especially pp. 103-108

BIOCHEMISTRY AND ENZYMOLOGY TEXTS

Boyer, Lardy and Myrback (Editors)
[illegible]
Academic Press, New York (1959)

Dixon and Webb
[illegible]
Academic Press, New York (1964)

Gutfreund, H.
[illegible]
John Wiley and Sons Inc., New York (1965)

Mahler and Cordes
[illegible]
Harper and Row, New York (1966)

Chapters 2, 3 and 7

[illegible]r, Todd, Mason, Van Bruggen
[illegible]
Macmillan Company, New York

Chapter 11, especially pp. 452-459

[illegible], Handler and Smith
[illegible]
McGraw-Hill Book Company, New York (1969)

Chapter 12

PERIODICALS

Phillips, David
"The Three Dimensional Structure of an Enzyme Molecule"
[illegible], Vol.215, #5 (November 1966)